Efficient dielectrophoretic cell enrichment using a dielectrophoresis-well based system

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Whilst laboratory-on-chip cell separation systems using dielectrophoresis are increasingly reported in the literature, many systems are afflicted by factors which impede "real world" performance, chief among these being cell loss (in dead spaces, attached to glass and tubing surfaces, or sedimentation from flow), and designs with large channel height-to-width ratios (large channel widths, small channel heights) that make the systems difficult to interface with other microfluidic systems. In this paper, we present a scalable structure based on 3D wells with approximately unity height-to-width ratios (based on tubes with electrodes on the sides), which is capable of enriching yeast cell populations whilst ensuring that up to 94.3% of cells processed through the device can be collected in tubes beyond the output. © *2013 AIP Publishing LLC*. [http://dx.doi.org/10.1063/1.4842395]

I. INTRODUCTION

In biological and clinical science, there is a significant need for the ability to separate large quantities of cells from a heterogeneous mixture. This could be for the separation of cancer cells from healthy tissues, or specific types of stem cells ahead of transplantation (such as those which will become neurons, required for treatment of brain damage), or those that respond to certain types of drugs in order to select cells for drug development. However, in order to perform such separations, we require both that a property exists that discriminates between the cells, and that a means exists to separate them on the basis of that discriminator. The most basic forms of filtration operate on the basis of particle size; particles are pushed through a filter and the smallest pass through the holes whilst the larger ones are trapped,[1] which requires cells that are significantly different in size and/or mechanical stiffness; a large cell will only be trapped if it is not able to deform and squeeze through the gap. Another commonly used technique for cell separation is fluorescently activated cell sorting (FACS), which uses fluorescent markers which differentiate the cell types (e.g., binding to proteins only present in the desired cell type), then firing cells serially through a laser and sorting them electrostatically into output streams. FACS systems are common but expensive, have high cell losses and relatively low throughput. Another method in common use is separation using magnetic beads precoated with an antibody to bind to antigens present on the surface of the required cells, which can then be collected following the application of a magnetic field; such systems are effective only where the cells are discriminated by the presence or absence of these surface markers.

Another method of cell separation is based on an electrokinetic technique called *dielectrophoresis*, or DEP. DEP is an electrostatic phenomenon of induced motion in particles such as cells in non-uniform AC electric fields; the magnitude and direction of this force is dependent

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on the properties of the particle and on the applied frequency, so that a frequency sweep can be used to elucidate the electrical properties of both membrane and cytoplasm of a population of cells.[2] As cells are particles which can be suspended for analysis, this means that the technique is eminently applicable to the manipulation, separation, and analysis of different cell types from one another, or from smaller particles such as viruses and bacteria.[2–4] Particles moved by dielectrophoretic forces can be made to exhibit a variety of motions including attraction to, and repulsion from, regions of high electric field (termed positive and negative DEP, respectively), according to whether they are more or less polarisable than the surrounding medium at the applied frequency. By varying the frequency, it is possible to produce a profile of the polarisability (a DEP spectrum) that can then be used to infer the electrophysiological properties of the cells.[5] Where the particles differ in properties such that different populations in a mixture respond differently to the field—for example, one experiencing DEP attraction, the other repulsion—those particles can be separated by DEP, with one population being retained from a flow by positive DEP whilst the other is repelled from the electrodes and washed away. If the field is then removed, the trapped particles are released and can be collected separately.

DEP separation has been of interest for many decades, first case having been described in 1966.[6] Since then, many researchers have developed systems to investigate the separation of circulating tumour cells from blood samples (e.g., Ref. 7); discriminate between, and subsequently separate, stem cell populations with different differentiation potential;[8–10] remove diesel particulate matter from airborne samples whilst retaining airborne bacteria;[11] as well as multiple separations of live and dead cells such as yeast.

In the 1990s, several studies were described where cancer cells were separated from blood samples by dielectrophoresis, though these typically had very low flow rates of a few microliters per hour; a clinical sample is typically of the order of an ml of blood, which could be considered a yardstick of clinical usefulness. Three publications have demonstrated throughput at rates high enough to be useful; the well-type device of Fatoyinbo *et al.*[11] was able to process 25 ml h^{-1}. The ApoStream system and related technologies[12,13] can separate at 7.5 ml h^{-1}, and the system presented by Hu *et al.*[14] could process concentrated cell flows at 0.3 ml h^{-1}. The latter two systems are based on microfabricated electrodes being driven by syringe pumps, but the Fatoyinbo design used a different fabrication method better suited both to industrial scale-up and mass production. Known as the DEP-Well system, the Fatoyinbo device used a laminate of conducting and insulating films (specifically domestic aluminium foil and epoxy resin) through which holes were drilled. This created chambers with electrodes "striped" around the perimeter, allowing a much higher DEP throughput; whereas other DEP-based separation system uses microfluidic channels to introduce cells, the DEP-Well system forced cell solutions through parallel wells to achieve much higher throughputs. For example, the first systems used 288 wells drilled in a 10 mm-radius circle.

In this paper, we present a novel optimized version of the DEP-well separation system using a novel sequence of wells in series, using gaskets to direct flow. Whilst laboratory-on-chip cell DEP-based separation systems using dielectrophoresis are increasingly reported in the literature, many are afflicted by factors which impede "real world" performance, chief among these being cell loss (in dead spaces, attached to glass and tubing surfaces, or sedimentation from flow), and high height-to-width ratio designs (large channel widths with small channel heights) that make the systems difficult to interface with other microfluidic systems. Here, we present a scalable structure consisting of 25 number of 3D wells with which is capable of enriching cell populations whilst ensuring over 90% of cells processed through the device can be collected in tubes beyond the output, whilst being scalable to multiple paths for significantly higher throughput.

II. MATERIALS AND METHODS

A. Cell preparation

Yeast cells (*Saccharomyces cervisiae*) were cultured in 2 g yeast extract-peptone-glucose (YPD) broth (Sigma Aldrich, UK) in 40 ml deionised (DI) water. The medium was sterilised in an

autoclave at 121 °C for 15 min. A small amount of yeast cells was scraped from a cultured colony on an agar plate using a sterile loupe and dipped into the prepared medium. The YPD broth medium containing the yeast cells was placed in the incubator for 18 h at 37 °C. Prior to experimentation, cells were centrifuged at 180 g for 3 min, washed 3 times, and resuspended, all of which used a medium containing 2.55 g D-mannitol in 50 ml DI water. Phosphate buffered saline was added to adjust the medium conductivity to 5 mS/m, verified using a conductivity meter (HI8733, HANNA instruments). To prepare non-viable yeast, cells were heated to 90 °C for 20 min before being resuspended as described above. Cell viability was assessed using the Trypan Blue test.

B. Well devices

Devices were developed from previous work on DEP-well devices.[15] The devices in this work were constructed from 5 layers of 40 μm-thick copper and 120 μm-thick polyethylene terephthalate (PET) adhesive tape, joined by a 24 μm-thick acrylic adhesive. The materials were arranged alternately and are drilled using a micro driller (model 395, Dremel) with high speed steel (HSS) tungsten carbide drill bits (RS components, UK) with 500 μm diameter to create 25 holes; when measured using a microscope and PhotoLite software, the average diameter of the wells was found to be 580 ± 26 μm. A typical device is shown in Figure 1(a). The distance between the centres of adjacent holes was approximately 2 mm. Wells were either drilled orthogonally to the copper surface, or at an angle of 35° to the vertical using a custom rig, as shown in Figures 1(c) and 1(d) respectively.

A photopolymer resin (Polydiam Industries Ltd., UK) was used to produce a gasket that also functioned as a flow channel. Gaskets were produced using a printed mask on transparency,

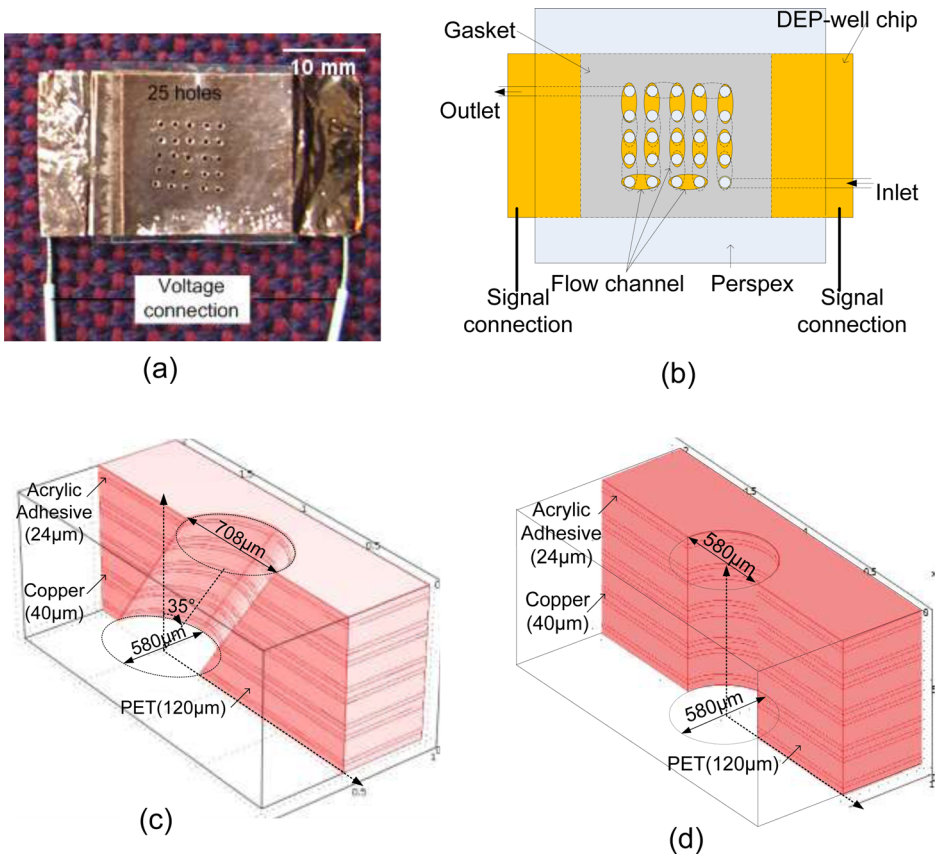

FIG. 1. (a) The 580 μm diameter of DEP-well consists of 25 holes that made of 5 layers of 40 μm-thick copper tape with 24 μm-thick acrylic adhesive and 4 layers of 120 μm-thick transparency plastic. (b) A schematic showing the gasket design and connections. (c) Cross-sectional view of a slanted well drilled at 35°. (d) Conventionally drilled well.

which was placed in a standard UV light box, followed by a plastic sheet and rubber spacers to set the thickness of the fabricated gasket to 500 μm. Polymer resin was poured over the mask and covered with a plastic sheet. The polymer was cured by exposure to UV light for 60 s; the uncured areas blocked by the mask were removed with soap in an ultrasonic bath. The gasket thickness was accurate to $\pm 37\,\mu$m when measured using a digital micrometer. Separate gasket designs were required for the top and bottom of the electrode device. A schematic showing the channel layout is shown in Figure 1(b).

C. Experimental setup

The trapping and recovery efficiency of the device was tested using yeast cells with a concentration of 5.2×10^6 cells/mL ($\pm 8\%$), which were suspended in a medium with a measured conductivity of 5 mS/m. The cells were loaded into a syringe and then were filled slowly into the device. The syringe was placed into a syringe pump and positioned vertically with the device in order to minimise cell sedimentation (Figure 2).

The yeast cells were first pumped through the wells without applying an electric field to produce a control profile that could be a reference line in determining the efficiency of cell trapping and recovery. To trap the cells, a signal was applied of $20V_{pp}$ amplitude at frequency of 1 MHz. The signal was changed to $10V_{pp}$ at frequency of 10 kHz to release the trapped cells. At the output, cells were collected directly into one of twelve Eppendorf tubes. A sample collector was constructed to facilitate the collection of sample in fractions. A unipolar stepper motor was used to rotate an Eppendorf holder to provide an empty tube at a specific interval time depending on the amount of volume needed to be filled in; for example, if sample is pumped at a flow rate of 5 μl/min, 360 s are required to fill up 30 μl of the sample in each Eppendorf before the motor rotates. The motor was driven by four NPN power Darlington transistors via parallel I/O port from the computer. Following the completion of the experiment, cell concentrations in the Eppendorfs were measured using a haemocytometer and microscope. The experiment was conducted with three different level of flow rates, namely, 5 μl/min, 10 μl/min, and 20 μl/min. The control experiment was only conducted with flow rate of 5 μl/min and 10 μl/min.

In order to assess the basic DEP behavior in the two well types (straight and angled wells), cells were observed moving in the presence of an electric field but the absence of flow, in a manner similar to well-based cell analysis devices described elsewhere.[15,16] As with conventional DEP-well based measurements, the magnitude of the DEP can be determined by observing changes in the light intensity across the well when measured from centre to edge. For each

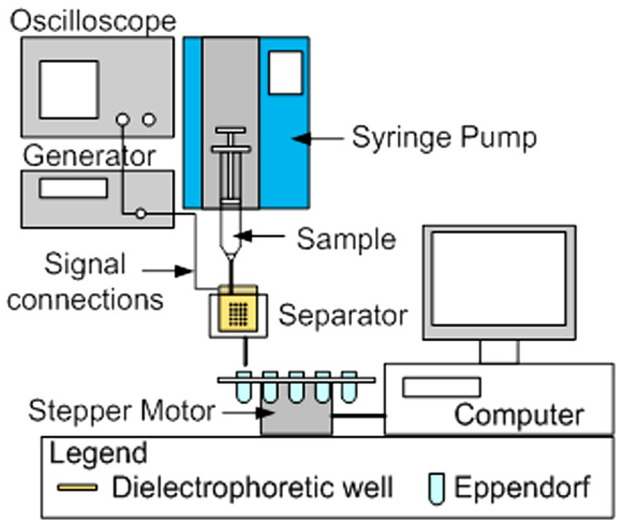

FIG. 2. The setup for determining the efficiency of the device.

well, yeast suspension at concentration of 5.5×10^8 cells/mL ($\pm 10\%$) was inserted with a syringe and covered by a thin glass slide to avoid light distortions. A custom cell characterization rig (shown in Figure 3) was fabricated, consisting of a standard DIN microscope objective lens with magnification of $10\times$ attached to a camera (WV-BL200, Panasonic) using a c-mount extension tube. The camera and electrode could be pivoted to allow observation of both the 0° and 35° wells. The well was then energized with $20V_{pp}$ at 1 MHz for 100 s. An image of the well was captured for every second by a camera and then processed using MATLAB to produce the changes of light intensity over time as a function of radius.[15]

III. RESULTS AND DISCUSSION

A. DEP behaviour in straight and slanted DEP-wells

When used in the characterization setup, the cells experienced positive DEP and were collected at the electrode edge, causing the cell concentration at the centre of the well to decrease. This was reflected in a higher light intensity at the centre of the well and a reduced intensity at the edge. A stronger DEP response created a larger change in light intensity along the regions. Figure 4 shows the activity of yeast cells in 0° and 35° wells, with and without the electric field applied. The data represent the average of five experiments. The x-axis represents the radius of the well, where number 1 indicates the centre of the well and 10 refers to the edge, and the y-axis represents the light intensity.

Without applying the electric field, a small change was detected in light intensity in the 35° well, which increased slightly at the centre of the well due to the sedimentation of the cells. When the electric field was applied, it was found that cells at the centre of 0° well were not influenced by the DEP force as no significant change in light intensity. This indicates that the cells at the centre were unable to be pulled towards the edge of well due to the symmetry of the system. However, by tilting the well, significant changes in light intensity were observed at the centre of the 35° well, indicating that larger numbers of cells were attracted by the DEP force towards the well edge.

On the other hand, the change of light intensity at the edge of 0° well electrode was greater than the slanted well, indicating that the DEP force produced at the edge of the slanted well is weaker than that in the straight well. The force is proportional to the strength of electric field but the magnitude of electric field is inversely proportional to the length between electrodes. When well was drilled with angle, the distance between electrodes along the axis becomes

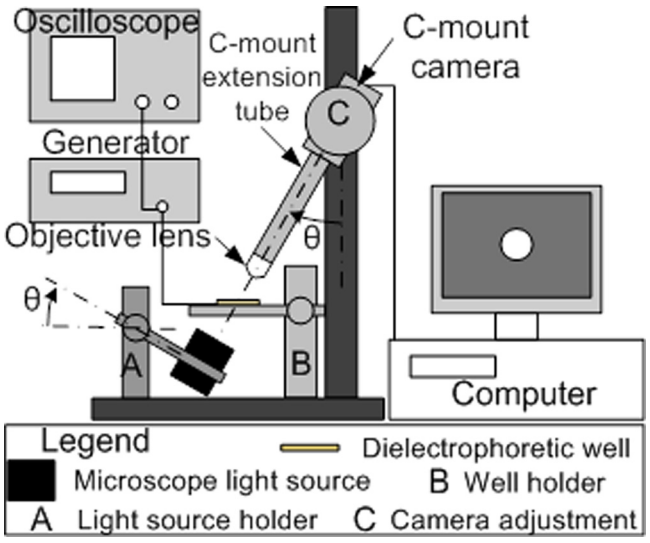

FIG. 3. The setup to observe the DEP force strength for different angle of well.

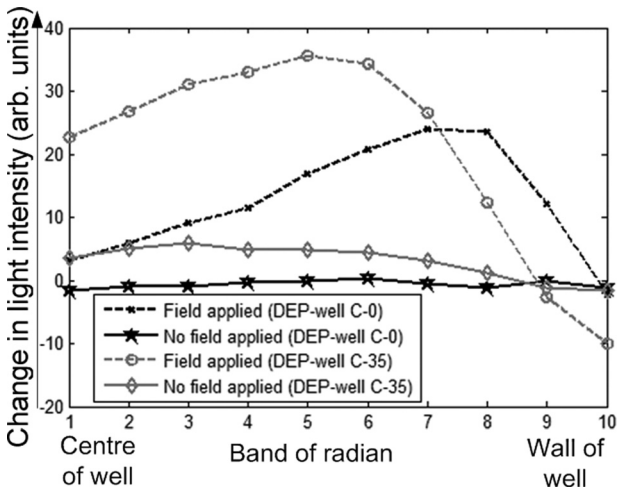

FIG. 4. The changes of light intensity in the well with angle of 0° and 35° over 100 s.

wider at the ends of the ellipse as viewed from above, reducing the strength of electric field and DEP force.

B. Cell trapping comparison between straight and slanted DEP-well

As described above, the output was collected in fractions, the size of which was dependent both on the volume of cell suspension to be delivered during the experiment, and the volume of the device itself which needed to be flushed through before cell collection would start. The total volume of the system was estimated to be approximately 80 μl. In order to collect the total output of the syringe plus the contents of the system before pumping within the 12 Eppendorf tubes at the output stage, the output of the device was sampled for every 40 μl. To calculate the cell trapping efficiency, Eq. (1) was used, where numbers 4 and 8 refer to the fraction numbers at the output during the trapping phase; the cell recovery efficiency was determined using Eq. (2)

$$\%\,cell\,trapping = \left(1 - \frac{\sum_{f=8}^{7} Rtr_f}{\sum_{f=4}^{7} Rc_f}\right) \times 100\,\%, \tag{1}$$

$$\%\,cell\,recovery = \frac{(Rtr_9 - Rtr_8) + \sum_{f=10}^{12}(Rtr_f - Rc_f)}{\sum_{f=4}^{8}(Rc_f - Rtr_f)} \times 100\,\%, \tag{2}$$

where f is the fraction number, Rc is the ratio of cells input when compared to the control sample (with no field applied), and Rtr is the ratios of cell output during the trapping period compared to the control sample. The first collection ($f=8$) after the signal was switched to $10V_{pp}$ at frequency of 10 kHz, was the number of untrapped cells that contained in the outlet tubing. Meanwhile, the cells collected in the fraction number 9 were the mixture of the untrapped cells and the trapped cells that have been released.

Yeast cells were used at a concentration of 6.2×10^6 cells/mL ($\pm 10\%$) to compare the trapping performance of straight and slanted wells. The well electrodes were placed in the

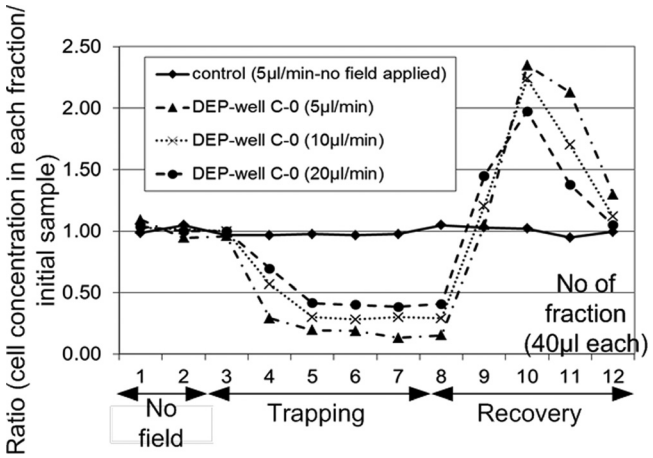

FIG. 5. The profile of yeast trapping and recovery using DEP-well C-0 (straight wells) at different flow rates.

separation device and a signal of $20V_{pp}$ at 1 MHz was applied to the well to trap the cells. The cells were trapped at three different flow rates namely 5 μl/min, 10 μl/min, and 20 μl/min. To recover the trapped cells, negative DEP was generated by applying $10V_{pp}$ at the frequency of 10 kHz and driven at 20 μl/min. A control experiment was conducted at flow rate of 5 μl/min, and then the flow rate was increased to 20 μl/min without applying the electric field throughout the experiment. All the experiments were repeated three times except for the control experiment. The profile of yeast trapping and recovery using the two well types are shown in Figures 5 and 6. Based on the profile, the percentage of trapping, recovery, and cell loss are calculated and tabulated in Table I.

The control experiment was conducted to assess the cell loss in the system in the absence of DEP. It was found that only 0.6% of cells were lost in the device. Later, the profile of the control experiment was used as a reference line in determining the percentage of trapping and recovery that obtained by the two devices when electric field was applied. Approximately, 80.5% of the yeast cells were able to be trapped at flow rate 5 μl/min using the straight DEP-well. The percentage was increased to 83.2% when 35° well was used. Similarly, when cells were pumped at 10 μl/min and 20 μl/min, the use of the slanted DEP-well to trap the cells produced 2.4% and 1.2% improvements over the straight DEP-well, respectively. These results proved that by tilting the well structure, more cells could be trapped. The difference of trapping percentage between 0° and 35° well was slightly larger when a lower flow rate was applied. The lower the flow rate, the

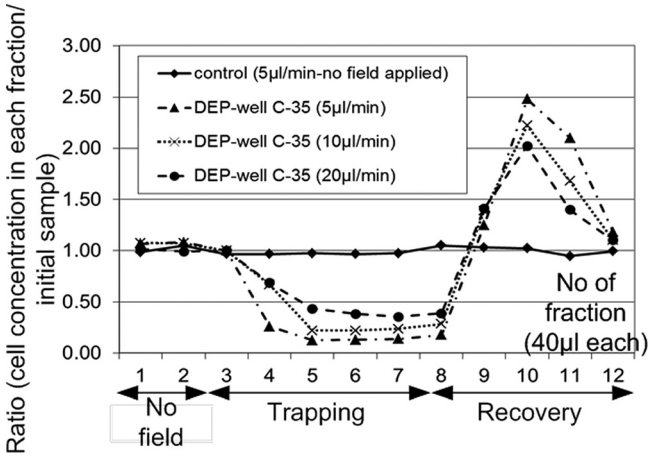

FIG. 6. The profile of yeast trapping and recovery using DEP-well C-35 (slanted wells) at different flow rates.

TABLE I. The performance comparison between the separation devices.

Experiment	Control	0° well			35° well		
Trapping field	No field	$20V_{pp}$ at 1 MHz	$20V_{pp}$ at 1 MHz	$20V_{pp}$ at 1 MHz	$20V_{pp}$ at 1 MHz	$20V_{pp}$ at 1 MHz	$20V_{pp}$ at 1 MHz
Trapping flow rate (μl/min)	5	5	10	20	5	10	20
Recovery field	No field	$10V_{pp}$ at 10 kHz	$10V_{pp}$ at 10 kHz	$10V_{pp}$ at 10 kHz	$10V_{pp}$ at 10 kHz	$10V_{pp}$ at 10 kHz	$10V_{pp}$ at 10 kHz
Recovery flow rate (μl/min)	20	20	20	20	20	20	20
% trapping	0	80.5 ± 2.1	64.6 ± 2.2	53.3 ± 2.7	83.2 ± 1.9	67.0 ± 2.1	54.5 ± 2.5
% recovery	0	93.5 ± 2.3	94.5 ± 2.0	94.6 ± 2.4	94.4 ± 2.2	95.1 ± 1.8	95.6 ± 1.5
% cell loss	0.6	10.1 ± 2.2	7. 9 ± 2.6	6.7 ± 2.8	8.4 ± 2.3	6.9 ± 2.5	6.9 ± 2.4

longer the residence time (t_r), thus allowing more cells near the centre of slanted DEP-well to be attracted towards the electrode and be trapped. Another potential contributing factor is that sedimentation in the slanted well may move cells from the center line to the areas of greater DEP force, though this might equally act against trapping of cells above the center line initially.

For the percentage of cell recovery, average of 94.6% of trapped cells were able to be released and collected after the electric field was changed to $10V_{pp}$ at 10 kHz. The frequency to release the cells was chosen to be 10 kHz based on the DEP-spectrum of the viable yeast, which produces negative DEP at that particular frequency. The attached cells were repelled and flushed out by the flow rate of 20 μl/min. The higher rate of cell loss (nearly 6%, rather than 0.6%) is possibly indicative of some cell adhesion to the electrodes after positive DEP.

C. Separation of non-viable and viable yeast

To test the device in separating and sorting two different types of cells, a 1:1 mixture of viable and non-viable yeast cells was prepared with total cell concentration of 6.5×10^6 cells/mL (±8%). This was suspended in a medium of conductivity of 5 mS/m and introduced into a slanted DEP-well device at flow rate of 5 μl/min. A 35° electrode chip was energised with $20V_{pp}$ at frequency of 1 MHz to trap the viable cells and separate them from the sample mixture, based on previous analyses of yeast DEP spectra.[16] The trapped cells were then released by switching the signal to $10V_{pp}$ at 10 kHz, producing a repulsive DEP effect and pushing cells from the electrodes into a 20 μl/min capture stream to obtain high recovery of the trapped cells. The output stream of the device was deposited into 12 Eppendorf tubes, each containing 40 μl as before. The numbers of viable and non-viable cells collected in each tube were counted using the hemocytometer. The non-viable cells were distinguished by adding the trypan blue solution. A ratio between the concentration of cells collected in each tube and the concentration of initial cell sample for both viable and non-viable cells was determined. The experiment was repeated three times to ensure the reliability of the system.

During the separation phase, 82.2 ± 3.5% of the viable cells were trapped when 5 μl/min was introduced. During the recovery phase, when the trapped cells were released in a 20 μl/min flow, the cells collected between 9th and 12th fractions produced 93.3 ± 2.7% of the trapped cells. However, the non-viable cells were observed to pass through the device unaffected during the separation phase, with only 5.7 ± 3.2% of the total number of cells introduced into the device was unable to be collected, meaning that 77% of the trapped cells and 94.3% of untrapped cells were collected in the output Eppendorfs, a rate comparable with separation strategies such as FACS and magnetic bead separation.

D. Simulation

In order to better understand the differences in trapping of the two well geometries, Comsol (Stockholm, Sweden) was used to perform the relevant simulations of trapping particles

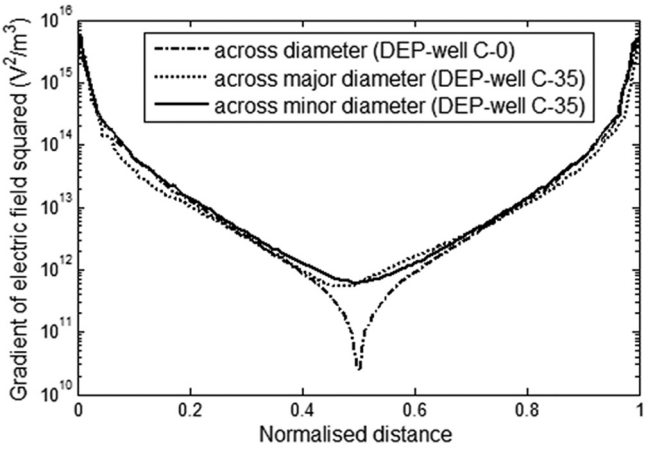

FIG. 7. The gradient of electric field squared across the DEP-well C-0 and the DEP-well C-35.

using DEP-well electrodes. The electrodes were modeled using full-3D modeling of the geometries in Figures 1(c) and 1(d), and the mean electric field was determined across the well. Since the 35° well is elliptical when viewed in the *x*-*y* plane, the mean across the major and minor axes were determined separately. The results are shown in Figure 7. As can be seen, the two geometries are similar in profile for the majority of the well diameter, albeit with the slanted well exhibiting a slightly higher field. However, the two geometries diverge significantly in the central 20% of the well; where the axisymmetric nature of the straight well means that the electric field at the centre of the well is zero, the broken symmetry of the angled well means that the field remains above zero at all locations. This small change—at a significant distance from the electrode—may nevertheless be sufficient to cause the small increase in trapping efficiency observed in the angled well devices.

IV. DISCUSSION AND CONCLUSION

We have presented an electrode design based on the DEP-well separation systems originally proposed by Fatoyinbo *et al.*[11] The basic concept is simple; through the use of gaskets on either side of the chip, the fluid flow weaves its way back and forth through multiple wells. This offers several advantages. First, the residence time in the separator is increased by the multiple passes through the chip. Second, the gaskets allows a pseudo-"mixing" step to be introduced; the different flow rates offers the potential for relocating cells within the bore, potentially moving them away from the centre where the force is weakest. Third, the design offers advantages over conventional 2D devices including low height-to-width ratios (assisting integration with other microfluidic components), and a significant lack of requirements for interconnecting tubing and dead space, meaning that the losses are remarkably small for such a device; the reported figures here represent typically 6% cell loss or less from initial solution to samples collected in Eppendorf tubes at the outlet. Fourth, the system is highly parallelizable; the original DEP-well separator device[11] used 277 wells in parallel. In the feasibility study described in this paper, only 25 wells have been used, connected in a series manner, but it would be a simple extension to parallelise several serial systems such as the ones described in this paper with common inlet and outlet manifolds constructed as part of the gasket; for example, a 250-well system (still smaller than that described by Fatoyinbo *et al.*[11]) configured as ten parallel routes of 25 wells in series would offer throughput ten times higher than that outlined in this paper. Furthermore, the devices constructed here were limited in the dimensions used by the available materials, and optimization of the geometry (particularly the electrode/spacer sizes and numbers of conducting layers) should yielded further improvements in the results.

We believe that both the scalability and the low value of cell loss during the separation process are important advantages of the device presented here. Whilst DEP separation offers

many advantages over other biological separation techniques such as FACS or magnetic beads, very little commercial development of the technology has taken place. There are many possible explanations for this, including low throughout, complexity of use, susceptibility of the system to the effect of bubbles, or loss of cells due to attachment to tubing or residence in dead space. Indeed, over the years many papers (not those cited here) have overpromised on DEP separation whilst not delivering consistent cell enrichment at biologically relevant throughput levels. Recent advances in separation technology by researchers around the world appear to be breaking through these limitations; we suggest that the scalability of the system presented here places it among the vanguard of these new developments.

In conclusion, the single well and multi-well electrodes were able to separate a mixture of viable and non-viable yeast cells when generating positive DEP force with the signal of $20V_{pp}$ at 1 MHz frequency. In practice, the system developed could be used to separate two mixtures of cells if the cells of interest exhibit negative DEP response which allows that cells to be collected with high purity.

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